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Elsevier Editorial System(tm) for Computer Methods and Programs in Biomedicine
Manuscript Draft

Manuscript Number:

Title: TUBERCULINE REACTION MEASURED BY INFRARED THERMOGRAPHY

Article Type: Full Length Article

Section/Category: Section II: Systems and Programs

Keywords: Tuberculine test; Infrared Thermography; Image processing

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Abstract: Setting: The infection with Mycobacterium tuberculosis gives a delayed immune response, measured by the tuberculine skin test. We present a new technique for evaluation based on automatic detection and measurement of skin infrared emission.
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Results: In 15 subjects, IR analysis was positive for tuberculine test. Mean temperature of injection area was higher, around 1 ºC, for the positive group (36.0± 0.3 ºC positive group; 35.2±1.7 ºC negative group, $p<0.001$, Non parametric U Mann-Whitney Test).
Conclusion: IR may represent an improved estimation of tuberculosis infection, given that it does not depend on reader variability and measures the increase of heat irradiation produced by the allergic tuberculine response.

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Conflicts of Interest

We declare no conflict of interests.

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25/2/2015

*Cover Letter

TUBERCULINE REACTION MEASURED BY INFRARED THERMOGRAPHY

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Running head: Tuberculine test measured by Infrared Thermography

Word Count: 3046

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ABSTRACT

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***Highlights (for review)**

Highlights

- First paper devoted to tuberculine reaction measurement based on thermal imaging.
- Proposed method alleviates the variability of a human supervisor
- Low cost system for solving a challenging diagnose

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INTRODUCTION

According to the World Health Organization, two billion people are infected with the tuberculosis (TB) bacilli[1]. It is estimated that every year eight million new cases appear. TB is a contagious disease not totally under control with great differences between rich and poor countries[2]. The diagnosis of TB is based on the interpretation of a skin immunologic reaction. The infection with *Mycobacterium tuberculosis* gives a delayed immune response, measured by the tuberculin skin test (TST)[3]. This consists of the intradermal injection of tuberculin purified protein derivative (PPD) and measurement of the resulting reaction. The induration size, measured in millimeters, indicates if the test result is negative or positive. Palpation and pen methods have been typically applied to measure the PPD reaction, but the measurement depends on the subjective interpretation of the reaction area[4,5]. New technologies are currently being developed to improve the sensitivity and specificity of the TB diagnosis[6].

In this study, we present a new technique for the evaluation of the tuberculine reaction based on the automatic detection and measurement of infrared (IR) emission of inflammatory effects produced by tuberculine immune response. IR thermal imaging is a noninvasive technique for monitoring temperatures and is widely applied in medicine[7,8]. The IR radiation increases due to inflammatory processes, such as that in the tuberculine reaction. An increased IR radiation is most likely caused by elevated blood flow, metabolic activity, and inflammatory reactions[9]. Therefore IR thermal imaging can be used to measure the rise in temperature due to an inflammatory process, which causes the increase of the blood flow with vascular dilatation, blood proteins and cellular extravasations[10] .PPD inflammatory response is characterized by edema, leukocyte lymphocyte-mononuclear infiltration, and erythrocyte extravasation in the dermis and epidermis[11]. Our hypothesis in the present work was that intradermal

inflammatory PPD reaction produced an increase in the temperature of the injected area, which could be measured by IR thermal imaging, and the features extracted from the resulting IR images after applying digital image processing techniques, helped to improve the reliability in the TB diagnosis.

MATERIAL AND METHODS

Ethics Statement

The study was conducted in the Respiratory Function Laboratory at HUGTIP, from February- September 2014, and approved by the hospital's Human Research and Ethics Committee. All participants came from pulmonology dept to discard tuberculosis contact and gave written informed consent, following the World Medical Association's Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects.

Mantoux tuberculin skin test

The Mantoux tuberculin skin test (TST) was performed in all subjects by intradermal injection of 0.1 ml of tuberculine purified protein derivative (PPD) (Tuberculina PPD Evans 2 UT/0.1 ml, UCB Pharma S.A., Madrid, Spain) in the anterior forearm. First, the reading of the Mantoux TST was manually performed 48 hours following the skin injection[3] by inspection, palpation, and measurement of the induration. An induration size greater than 0.5 cm was considered to be a positive TST result. A technician with more than 20 years experience in TST lecture has made all TST test[12].

IR thermal imaging

The size of the induration was also measured by IR thermal imaging. Subjects remained in the laboratory room for at least 15 minutes to achieve thermal equilibrium. Temperature, humidity and air circulation were all controlled. Laboratory temperature was maintained between 23 ± 1 ºC, and humidity was around 50%. Subjects were requested not to consume hot drinks or food for at least an hour before image session, nor use any skin preparations such as creams or talcum powder. Emissivity of the skin

was assumed at a value of 0.98 ± 0.01. There was no ceiling air filter. The lighting in the laboratory was maintained at wavelengths longer than 1 micron.

The IR thermal images were obtained from the anterior surface of the forearm of each subject, using a TiR32 Fluke Camera (2009 Fluke Corporation USA; 320x240 Focal plane array; 0.01 Cº Noise Equivalent Temperature Difference; 8-14 µm spectral range). The IR thermal camera was installed on a tripod with a fluke compact photo-movie. The distance between the camera focus and the forearm was 40 cm. The forearm was placed on a black foam cushion over a wooden table, with the anterior surface facing the camera focus. To have a reference distance, we placed a 24 mm diameter coin below the area to be measured. The detection of that reference object allowed us to convert pixels to millimeters (Figure 1). The image acquisition and storage were made with the SmartView® software. Each image was exported in JPG format. Moreover, the temperature measurements were transferred to a text file.

IR image processing

The IR image processing was performed using Matlab 2014b and following the scheme shown in Figure 2. Each text file containing the temperature data of an IR image was imported in a 2D numerical array (*temp*). As IR images had different temperature ranges, each *temp* array was scaled, as in (1), in order to have values between 0 and 1, thus obtaining the gray scale image *temp_n* (Figure 3-a).

$$temp_n = \frac{temp - T_{min}}{T_{max} - T_{min}} \quad (1)$$

T_{min} and T_{max} are the minimum and the maximum temperatures in *temp* respectively. After obtaining the gray scale image *temp_n*, we detected the reference object of known size that was present in the image in order to obtain the conversion

factor from pixels to millimeters. For that purpose, we first applied the Canny edge detector to the normalized image *temp_n* (Figure 3-b). Then, since the reference object was a circle, we used the circular Hough transform to detect its position and match its edge (note that the Hough transform can be adapted to detect specific shapes in an image)[13]. As the radius in millimeters of the reference object was known, we were able to calculate the conversion factor to express measured lengths in millimeters (Figure 3-c).

The temperature in the forearm area was much higher than the temperature outside the forearm area (air). Therefore, the values of all pixels in the forearm area were in the upper range of the scaled *temp_n* image. In order to clarify the differences between the internal pixels of the forearm area, we applied an intensity transformation to *temp_n*. All pixels below 0.5 were set at 0, whereas pixels in the range 0.5-1 were expanded to the range 0-1, thus obtaining the *temp_e* image (Figure 3-d). The difference between the PPD reaction area and the rest of the forearm was more noticeable in *temp_e* than in *temp_n*.

The segmentation algorithm searched two different regions: region 1 (PPD reaction area) and region 2 (erythema). A region growing method was used for the image segmentation. Assuming that the PPD reaction area (region 1) was near the image center, the first seed pixel was the pixel with the maximum temperature (P_{Tmax}) inside a centered rectangle area. A binarization was applied to the image *temp_e*. Pixels of the surface of the forearm were retained (set at 1) and pixels outside the forearm (air) were rejected (set at 0), thus obtaining the *temp_b* binary image (Figure 3-e). Then, a 5x5 cm square, which was centered at P_{Tmax}, was selected as the analysis area mask.

Having found the first seed point and defined the analysis area, the region growing algorithm was applied to *temp_e* by means of the following iterative procedure until convergence to a fixed point was reached. At each iteration, new sub-regions 1 and 2 were grown from a new seed point, by adding in neighboring pixels according to each sub-region membership criterion. First, sub-region 1 was grown by adding in neighboring pixels that met the criterion in (2).

$$-A * Th1 < \min S_1(i,j) < Th1 \ \rightarrow S_1(i,j) = reg_mean_1 - temp_e(i,j) \qquad (2)$$

The parameter reg_mean_1 was the mean value of all pixels included in the whole region 1, *temp_e(i,j)* was the intensity value of a neighboring pixel, $S_1(i,j)$ was the membership criterion of a neighboring pixel, *Th1* was the threshold used to either include or not a neighboring pixel in sub-region 1, and *A* was the scale factor of *Th1* to define the lower limit of $S_1(i,j)$. When there were not neighboring pixels meeting the criterion defined in (2), a new criterion, as defined in (3), was used to add in neighboring pixels to sub-region 2.

$$\min(|S_2(i,j)|) < Th2 \ \rightarrow S_2(i,j) = reg_mean_2 - temp_e(i,j) \qquad (3)$$

The parameter reg_mean_2 was the mean value of all pixels included in the whole region 2, *temp_e(i,j)* was the intensity value of a neighboring pixel, $S_2(i,j)$ was the membership criterion of a neighboring pixel, and *Th2* was the threshold used to either include or not a neighboring pixel in sub-region 2. When no neighboring pixels met the criterion defined in (3), a new seed point was selected and a new iteration started. Each new seed point had to meet the criterion defined in (2) and be at a maximum distance of 15mm from the sub-region 1 obtained in the first iteration.

After the region growing process finished, when no new seed points existed, regions 1 and 2 were formed by a set of sub-regions 1 and 2, respectively (Figure 3-f). The maximum length of both region 1 and region 2 were calculated as the sum of the maximum length of their corresponding sub-regions (Figures 3-g and 3-h). The background area was calculated by removing the pixels belonging to either region 1 or region 2 from the analysis area mask. Then, the area (mm^2), the minimum, the maximum, the mean, and the standard deviation temperatures (^oC) were calculated either for region 1, region 2, and the background.

Finally, the region 2 was merged with the background if the following criterion was met:

$$t_{mean2}\text{-}t_{std2}\text{-}t_{mean3}\text{-}t_{std3}<=0.3 \quad (4)$$

where t_{meank} and t_{stdk} (k = 2,3) were the mean and standard deviation temperatures of region 2 and background, respectively. Furthermore, region 1 was merged with region 2 and the background if criterion defined in (4) and the following criteria were met:

$$t_{mean1}\text{-}t_{std1}\text{-}t_{mean23}\text{-}t_{std23}<=0.25 \quad (5)$$

$$t_{mean1}\text{-}t_{std1}\text{-}t_{mean2}\text{-}t_{std2}<=0.3 \text{ or } t_{mean2}\text{-}t_{mean3}>=0 \quad (6)$$

where t_{meank} and t_{stdk} (k = 1, 23, 2, or 3) were the mean and standard deviation temperatures of region 1, region 2+background, region 2, and background, respectively. In those cases, only a background area was obtained as the final result of the image segmentation. Therefore, no reaction area was found and the test was negative. A negative/ positive segmentation indicated an IR negative/ positive result respectively. Also, for an expert in IR imaging that made all lectures of patient IR image, the non

existence of significant geometric IR color differences in the injection area was classified as a negative result.

Statistics results were made by means of SPSS soft. Parametric and non parametric tests were applied to observe the differences between groups. A Bland-Altman plot and the Kappa Statistic were also applied to see the inter-observer variation.

RESULTS

Table 1 expresses the anthropometric characteristics of 21 subjects (7/14 M/F) suspected of tuberculosis contact. Mean age was 57(17.9) years. Six had a positive PPD lecture (more than 5 mm of papule). In 15 subjects IR analysis was positive for tuberculine test.

Positive reaction of the IR visual examination was identified as one or more central geometric images with circular, elliptic or with more irregular contours, easy to differentiate from the rest of the IR surface (Figure 1). A higher temperature of this area appeared with a more intense color respect to the neighboring (blue to red colour scale). In subjects with a negative PPD test these characteristics were not evident in the IR image. Only one subject with negative PPD reaction presented a IR positive characteristic image.

Table 2 depicts IR parameters for the two groups of subjects: Group 1 with a positive IR image (15 subjects) and Group 2 with negative IR reaction (6 subjects). Mean, min, and maximum temperatures were higher for group 1 than group 2. Differences for mean temperature were around 1 ºC between both groups. These parameters were higher in area 1 than area 2 for group 1 and there were not significant differences from group 2. Differences for group 1 were around 0.5 ºC between area 1 and area 2.

Regression coefficient between diameter of area 1 measured by exploration and IR analysis was R=0.8, R^2=0.64, constant =6.13, coefficient beta=0.8.p<0.001. for all subjects with a positive PPD (14). Mean difference of diameter measured by PPD examination and IR analysis was around 1.4 mm (-12.6 to 5.5).

A Bland-Altman plot compares two assay methods. It plots the difference between the two measurements on the Y axis, and the average of the two measurements on the X axis (figure 4). Differences between diameter of induration measured by exploration of PPD and IR image analysis of area 1 were into the 95% limits of agreement (mean bias plus or minus 1.96 times its SD). The bias is computed as the value determined by one method minus the value determined by the other method. Bias was 1.4. and SD of Bias was 5.25. 95% limits of agreement were from -8.9 to 11.7.

Inter-observer variation was measured by The Kappa Statistic, between exploratory lecture of PPD and IR image analysis (Table 3). The agreement was 0.89 (almost perfect 0.81-0.99. Kappa Index).

DISCUSSION

The authors studied the skin tuberculine reaction in 21 tuberculosis contacts by infrared emission image analysis and compared it with classical tuberculine examination. Fourteen subjects with a tuberculin reaction size more than 5 mm had an IR positive image reaction. Six subjects with negative tuberculin test had also a negative IR image. One contact with a negative tuberculin test had a positive IR image. The diameter of the IR reaction was higher than the diameter measured by skin examination.

Infrared imaging has been extensity applied in medicine since the 1960's. Detection of peripheral vascular disorders and breast cancer are some examples[7,14,15,16,17]. In areas affected by a inflammation process characterized by arteriolar capilar and venula dilatation with inflammatory infiltrate there is an increase in an increasing of eat[10]. IR is able to detect skin temperature differences as low as 0.1

C°[18]. These properties had been applied in several medical cases as control of bar infection[19], replacement of total knee[20] and acne treatments[21,22].

The current measurement of tuberculine reaction is the subjective measure of skin induration by tact. However, the reading of induration could be a potential error source[5]. Margins of the induration are difficult to find independently of technique (ballpoint-pen, palpation)[4]. On the other hand, it could be a subcutaneous inflammation due to the tuberculine reaction but not evident by palpation as a false negative result or doubtful lecture[4]. In this case, skin IR emission could be more sensible that a simple examination. Histopathologic pattern of human intracutaneous tuberculine reaction was described by Kuramoto[23] and more recently by Haholu[11] .These reactions take place in the epidermis and dermis. These cellular infiltrations with an extensive vascular component (vasodilatation, edema exudation) are responsible for an increase in the heat generation on the injection place that can be measured by IR camera[10]. Changes from 0.2 to 2 ºC have been demonstrated in inflammatory dermatologic processes[20,24,25].

We observed a significant difference in the mean temperature between the central segmented IR area and window hand area at around 1 Cº. This temperature was above that measured in healthy subjects[8]. In addition, the difference between the central and surrounding area temperatures was significant, around 0.5 Cº. A higher production of heat indicates different inflammatory events in the same way that the small indurations tact is different from the skin eritema area. Infection with M tuberculosis causes a cell-mediated immune response leading to sensibilized T lymphocyte[26]. Intradermal injection of PPD evokes a delayed hyper sensibility response mediated by sensitized T cells that produces a skin induration[23]. Erithema multiform and spongiotic dermatitis are more related with PPD score based on the induration area. The most

frequent, basal spongiotic dermatitis, is characterized by edematous changes in the epidermis and mononuclear exocitosys. In addition, the erythema multiform type has an important component of edema with erythrocyte extravasations. In consequence we believe that marked edema as a consequence of a prolonged vasodilatation with cellular extravasations are responsible for increased temperature in the central area. This fact is shown by an increase of irradiated heat as observed in other inflammatory diseases[10].

One important finding of the current work was that one subject with a negative PPD had a positive reaction to the IR analysis. The possibility that PPD shows false negatives has been previously reported[27]. An immune compromised state (HIV infection) or inmunologic-suppression and booster phenomenon may lead to false negative results[27]. Methodological technical causes such as sub-dermic, superficial injection with an easy rupture of the vesicle, as well as a subjective interpretation caused by inter-reader variability could constitute other causes of false negative results[5]. In fact, there is a 10% rate of false negative PPD's in hospitalized patients, increasing to 50% in cases of disseminate tuberculosis[26].In consequence, IR could substantially improve the sensibility in these cases. We are not able to add any new data regarding false positive cases, which is an intrinsic problem of the tuberculine reaction. IR only provides information about local skin inflammatory reactions regardless of the kind of inflammation or germ-causing disease. New immune based tests such as interferon-γ release assay or POC technologies could increase the sensibility and specificity of tuberculosis diagnostic[6].

Several issues related to methodology of the present paper must be addressed. First, the reduced group of patients has not permitted us to draw general conclusions, but in the present work a positive IR reaction was observed in all TST manual lecture

except in one and also negative IR reaction was observed in all negative TST test. In conclusion, although it needs a larger sample to extract definitive conclusion, we thing that these results induce to perform a multicentre study that will permit the definitive generalization of present procedure. On the other hand, infrared reading was made following the guidelines for standardization in Medical Thermography[17]. We think that this procedure could applied with no restrictive conditions that will permit to use more simple elements as an IR mobile-camera in different spaces for its general use.

Worth to mention that our previous work[28] revealed that thermal imaging provides complementary information to visual imaging, from an information theory point of view.

Conclusion

IR may represent an improved estimation of tuberculosis infection, given that, IR lecture does not depend on reader variability and only measures the increase of heat irradiation produced by the allergic tuberculin response, which is the IR physical principle that could be objectively measured.

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TABLE 1 ANTROPHOMETRIC CHARACTERISTICS OF SUBJECTS WITH SUSPECTED TBC

	Male			Female			
(M/F)	Age(ys)	Height(cm)	Weight(Kg)	Age(ys)	Height(cm)	Weight(Kg)	Total
All	57.0(17.8)	156.0(17.4)	70.0(20.1)	51.4(15.9)	155.8(8.1)	52(11.6)	21
Positive (6/9)	48.3(18.7)	172.5(5.9)	73.1(10.0)	45.2(13.8)	159.3(6.4)	64.9(8.7)	15
Negative (1/5)	32	189	97	62.4(14.3)	149.4(7.3)	64.7(16.9)	6

Table 1. Anthropometric values of 21 subjects with tuberculosis contact suspected . Positive and negative test responses were measured by infrared analysis (IR) with a term graphic IR camera. All a values are in mean(sd).

TABLE 2. INFRARED LECTURE OF SUBCUTANEAU TUBERCULINE TEST IN PATIENTS WITH SUSPECTED OF TUBERCULOSIS

Group 1 (+) (15)	Area 1	Area 2	Area 3
Area mm^2	0.96 (0.75)*+	6.71 (7.88)	17.17(7.76)
T min ºC	35.93(0.85)*+	35.33(1.06)	33.52(1.11)
T max ºC	36.34(0.78)*+	36.02(0.83)	35,82(1.08)
T mean ºC	36.04(0.33)*+	35.57(0.96)	34,71(0.93)
Diameter mm	15.45(7.24) +	37.45(20.53)	
Group 2 (-) (6)	Area 1	Area 2	Area 3
Area mm^2	7.54 (5.31)	5.71 (2.91)	6.27 (3.59)
T min ºC	34.27(3.45)	34.17(1.77)	32.78(2.00)
T max ºC	35.15(1.51)	35.07(1.61)	35.73(1.46)
T mean ºC	35.18(1.68)	34.51(1.67)	34.58(1.48)

- * Signification level $p<0.05$. Non parametric U Mann-Whitney Test between Groups
- + Signification level $p<0.05$. Difference between area 1 and 2. Non parametric Wilcoxon Rang-sig test for the same group.

Group 1:Tuberculine lecture IR positive ; Group 2: Tuberculine lecture IR negative.

All values are in mean(sd)

TABLE 3. INTEROBSERVER AGREEMENT. TheKappa Statistic

		Exploratory lecture of PPD		
		Positive	**Negative**	**Totals**
IR image	**Positive**	**14**	**1**	**15**
	Negative	**0**	**6**	**16**
	Totals	**14**	**7**	**21**

Kappa Statistic agreement between observed PPD exploration and IR image analysis in 21 subjects with TBC contact suspect.

Absolute agreement= 0.95. Hope agreement= 0.57.

Kappa Index: 0.89. Standar error I.C 95% (0.67-1.1).

FIGURE 1-IR images in a positive PPD test.

Figure 1-1 is the photograph of the injection tuberculine area. We can observe a higher IR intensity in the area of tuberculine injection (Figures 1-1, 1-3). Figure 1-2 is a superposition of IR and colour photographic image. To have a reference distance, we placed a 24 mm diameter coin below the area to be measured

FIGURE 2 – Flowchart of the IR image processing algorithm.

First, image *temp* was scaled, thus obtaining image *temp_n*. Then, the reference object was detected (center and radius), which allowed us to calculate the conversion factor from pixels to millimeters. Next, an intensity transformation was applied to *temp_n*, thus obtaining image *temp_e*. A region growing segmentation algorithm was applied to *temp_e* in order to obtain region 1, region 2, and the background. Finally, some parameters are calculated for each region and these parameters were used to decide if some region were merged or not.

FIGURE 3-Different aspects of the image processing algorithm

(a) *temp_n* image; (b) Edges detection by Canny's method; (c) *temp_n* image after rejecting the internal pixels of the reference object; (d) *temp_e* image (white expansion) and the analysis area (red square); (e) Analysis area mask; (f) PPD reaction area (white), erythema (light gray), and background (dark gray); (g) PPD reaction area and its maximum distance; (h) Erythema and its maximum distance; (i) Edges of the PPD reaction area and the erythema.

FIGURE 4. Bland-Altman plot

A Bland-Altman plot compares two assay methods. It plots the difference between the two measurements on the Y axis, and the average of the two measurements on the X axis. In this case the two measures are: the diameter measured by manual exploration of the PPD papula and the diameter of area 1 measured by IR image analysis. The 95% limits of agreement are shown as two dotted lines.

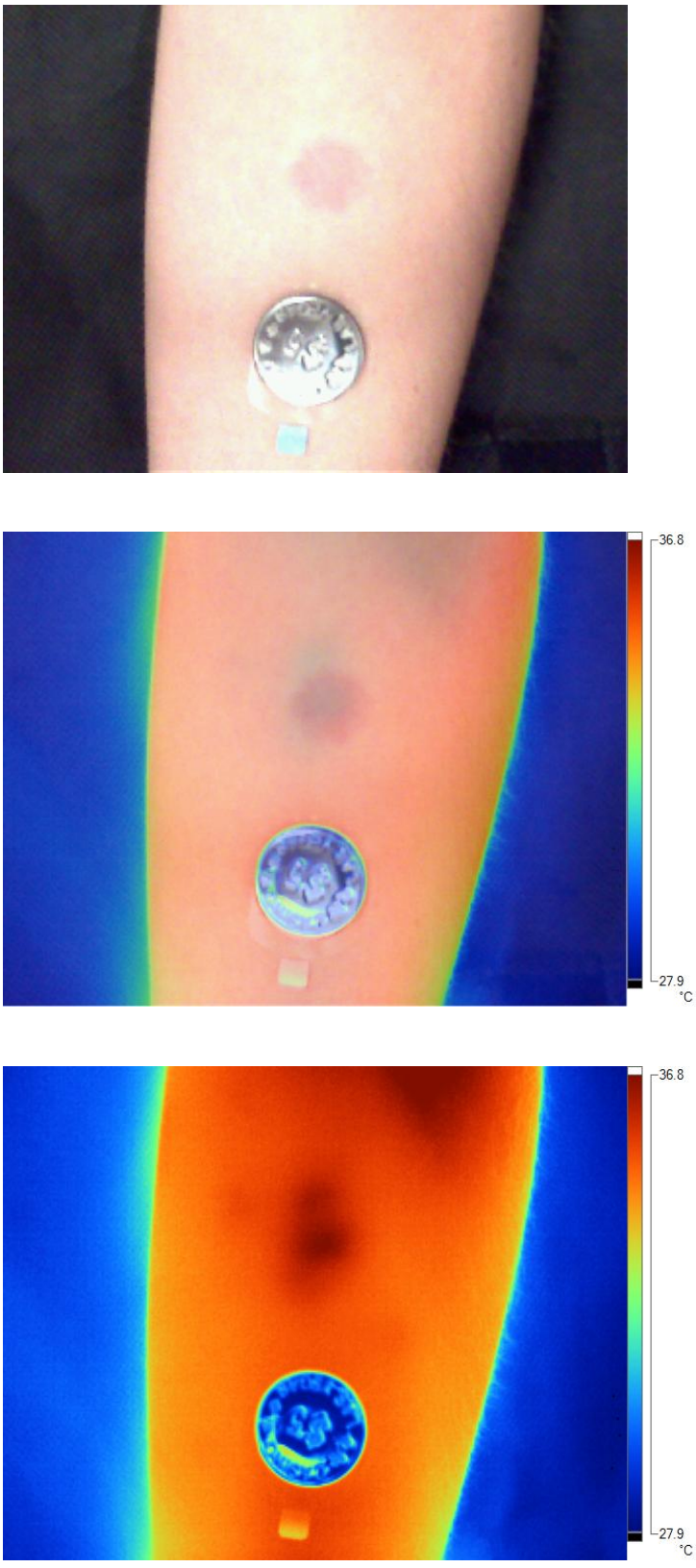

FIGURE 1.IR images in a subject with a positive PPD test

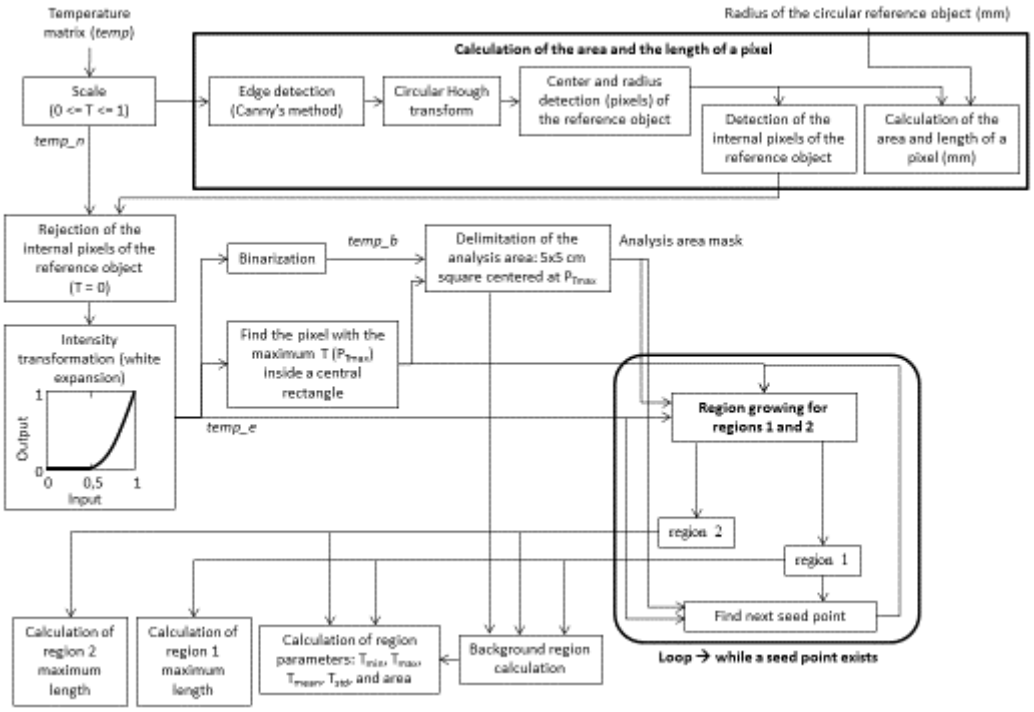

FIGURE 2 . Flowchart of the IR image processing algorithm

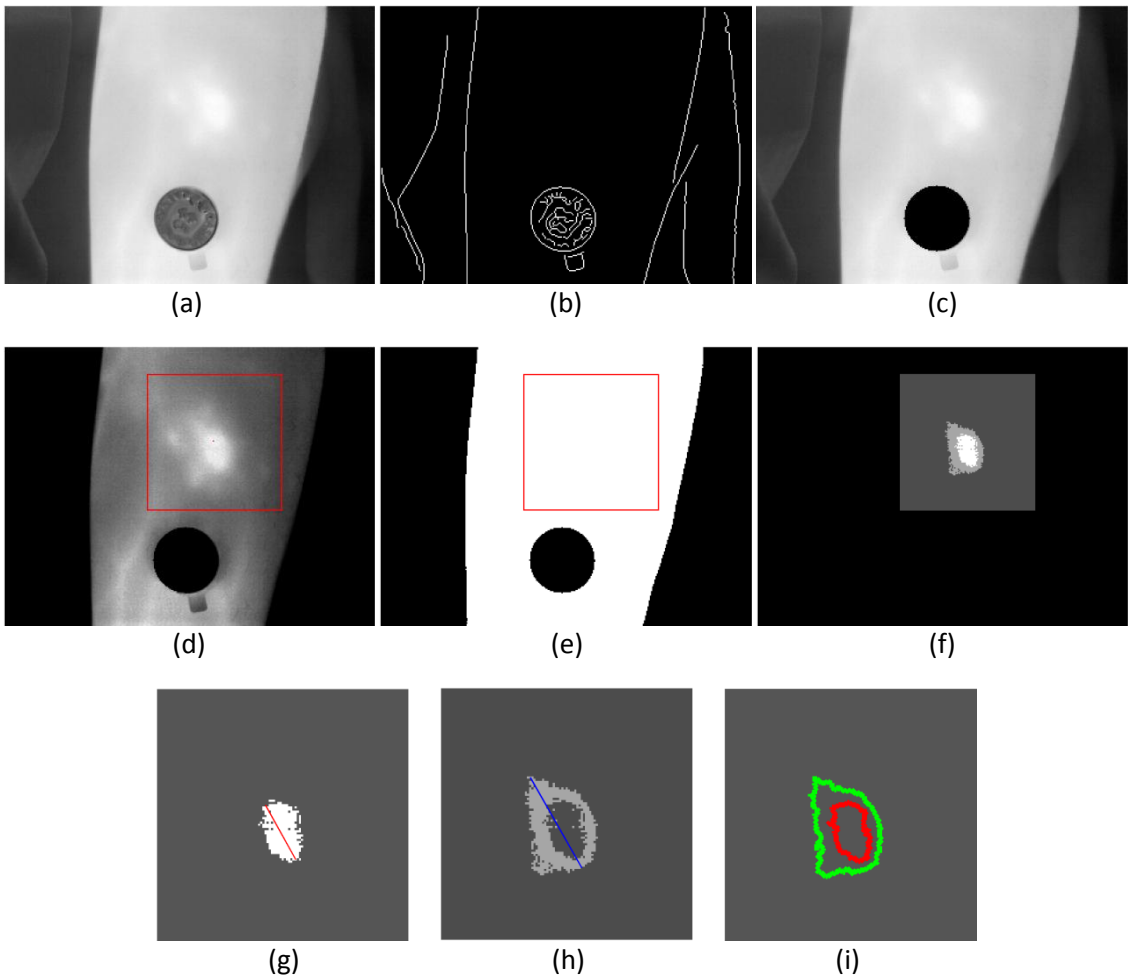

FIGURE 3. Different aspects of the image processing algorithm

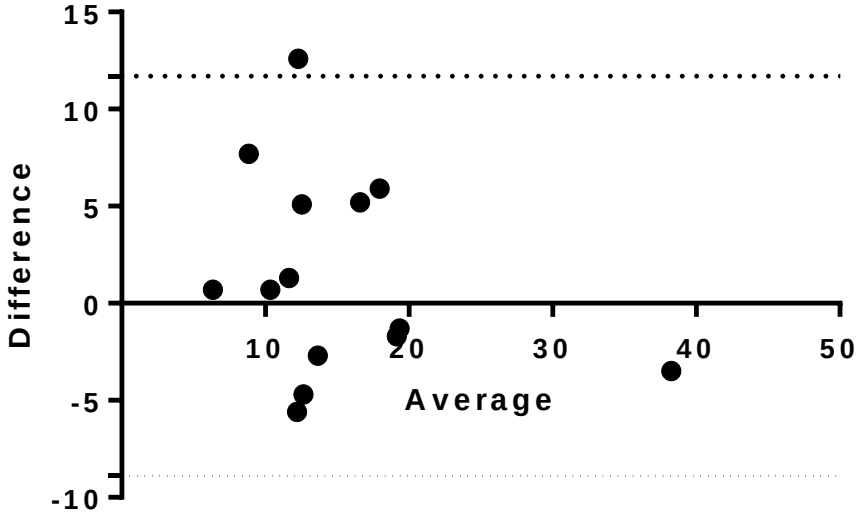

FIGURE 4. Bland-Altman plot

Declaration of conflict of interest

The authors state that they have no conflict of interest in the elaboration of this paper.

Acknowledgements

The authors wish to thank the nurses Rosa Gomez and Aurora Torrents for their special assistance.

AUTHOR'S CONTRIBUTIONS:

- José Antonio Fiz Fernández: inventor of procedure, design of the work; processed and automation of image analysis; analysis and interpretation of the data for the work; writing the article; guarantor of the paper.
- Manuel Lozano :Processed and automation of image analysis; analysis and interpretation of the data for the work; writing the article
- Enrique Monte Moreno: Analysis and interpretation of the data; writing the article; revision of the word.
- Marcos Faundez: IR support and revision of the word.
- Laura Rodriguez Pons: Clinical study of patient Inclusion
- Caroline Becker: Clinical study of patient Inclusion.
- Adela Gonzalez_Martinez: PPD test realization.
- Juan Ruiz Manzano. :Clinical study of patient Inclusion.